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Changes in body fluid compartments, tissue water and electrolyte distribution, and lipid concentrations in rhesus macaques with yellow fever

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SUMMARY

Rhesus macaques were inoculated subcutaneously with 40 plaque-forming units of yellow fever (YF) virus. To identify pathophysiologic mechansisms of YF, rectal temperatures and evidence of viremia were observed daily; physiologic and biochemical changes were studied on postinoculation day (PID) 5. Marked viremia was detected on PID 2 through 5, and fever was first observed on PID 4. On PID 5, blood and plasma volumes and circulatory K^+ values increased, whereas RBC volume, PCV, and plasma cholesterol concentration decreased. Total lipids (mainly triglycerides) accumulated in the liver of inoculated macaques; alterations in hepatic content of water, electrolytes, and trace metals were also observed. Certain parts of the CNS, skeletal muscle, skin, heart, diaphragm, and renal cortex were affected, with changes noticed in water, electrolyte, trace metal, and lipid concentrations. These tissue changes indicated that cellular metabolism was altered and that the transport mechanisms of cell membranes of certain tissues were modified by YF virus or the disease process caused by the virus. Terminal hypoglycemia (57.6 ± 12.1 mg/dl) was observed. The YF-induced intracellular dehydration of the medulla oblongata at the later stage of illness may depress the cardiovascular and respiratory centers, thus contributing to death of rhesus macaques infected with YF virus.

Early studies of yellow fever (YF) virus infection have focused mainly on pathologic and immunologic assessment of the disease process,[1-4] with few investigations reported on physiologic and chemical responses.[5-8] Encephalitis,[9] renal impairment, cardiac dysfunction, and liver necrosis with fat infiltration were shown in human beings[5,8] and rhesus macaques[5,7,10] with YF. Other findings included accelerated metabolism of L-thyroxine and increased total activity of serum lactate dehydrogenase.[11,12] The main objectives of the present study were to identify disease-producing mechanisms of YF and possible causes of death and to study body fluid compartments, tissue water and electrolyte distribution, and trace metal and lipid concentrations at a late stage of YF in rhesus macaques.

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Materials and Methods

The study was performed in a closed suite; facilities were designed to permit a class III level of microbiologic containment.[13]

Fifteen healthy male rhesus macaques weighing 3 to 5 kg were assigned to a control (5) or an experimental (10) group. All macaques were caged individually, and food and water were provided ad libitum. A 6-ml control blood sample was obtained from each macaque before inoculation. Blood (5 ml) was incubated with ^{51}Cr (15 μCi) at room temperature for 30 minutes to label the RBC.[14] After plasma was removed following centrifugation, labeled RBC were washed with isotonic saline solution 3 times and were suspended in 5 ml of saline solution. Blood volume was determined by injecting 4 ml of radiolabeled RBC suspension according to the dilution principle.[14] The remaining 1 ml of blood was centrifuged at 3,500 × g for 10 minutes. Plasma was separated and used for determinations of glucose[15] and YF antibodies.[16]

Ten macaques with no detectable YF antibodies were inoculated subcutaneously with approximately 40 plaque-forming units (PFU) of YF virus (Asibi strain). Control macaques were given isotonic saline solution in a similar manner. Rectal temperature (using a clinical thermometer) and body weight were monitored daily; blood samples were obtained by direct femoral puncture on postinoculation days (PID) 2 to 5 for viremia determination.[16] By PID 5, when death appeared imminent in the inoculated macaques, the experiments were performed. All 15 macaques were anesthetized with ketamine (10 mg/kg of body weight), and the femoral artery and vein were cannulated. Several indicators, including tritiated water, sodium thiocyanate, Evans blue dye, and ^{51}Cr-labeled RBC were injected into the circulation via the femoral venous catheter; a series of blood samples was drawn at different intervals from the arterial catheter for determinations of various body fluid compartments, including plasma volume, RBC volume, extracellular water, and total body water.[14] Blood volume, interstitial water, and intracellular water were calculated.[14] Identical experimental procedures were applied to control and infected groups of macaques.

After completion of measurements of body fluid compartments, a larger arterial blood sample was drawn, and a thoracotomy was performed. Within 5 minutes after arterial circulation was interrupted, 12 tissues, including liver, lung, left ventricular muscle, renal cortex, renal medulla, diaphragm, skeletal muscle, cerebral cortex, cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord were excised.

Eight of the 10 experimental macaques survived and were euthanatized. The water and total lipid content and concentrations of electrolytes (Na^+, K^+, and Cl^-), minerals (Ca^{++} and Mg^{++}), and

trace metals (Zn^{++}, Cu^{++}, and Fe^{++}) were determined for each tissue sample. The Na^+ and K^+ concentrations of tissue extracts in 10% trichloroacetic acid were determined on a flamephotometer. Tissue Cl^- extract in distilled water were measured with an automatic digital chloridometer. Concentrations of tissue minerals and trace metals were measured by an atomic absorption spectrophotometer after each tissue (approx 1 g) was digested in 1 ml of 25% tetramethylammonium hydroxide. Distribution of tissue water, electrolytes, and minerals were calculated.[17] Detailed procedures for the determination of tissue water and electrolytes in samples from rhesus macaques were previously reported.[18] Plasma concentrations of electrolytes (Na^+, K^+, and Cl^-) were determined, using the procedures identical to those used for tissue extracts. The total amount of circulating electrolyte was calculated as electrolyte concentration × plasma volume.

Total lipids in plasma and tissues were extracted with chloroform-methanol mixture (2:1, v/v) for approximately 20 hours at room temperature.[19] A 20/1 ratio of solvent to minced tissue sample was used; the lipid extract was purified.[20] The weight of total lipids in each tissue was measured gravimetrically after complete evaporation of the solvent from the purified extract. The determination of triglyceride was based on the quantitative measurement of the glycerol moiety,[21] as modified by Newman et al.[22] Phosphorus content of phospholipid was determined.[23] The phospholipid concentration was obtained by multiplying the lipid phosphorus value by a factor of 25. Total cholesterol was also analyzed.[24]

Statistical comparisons of the data were made and SEM were calculated. Mean values of control and infected groups were compared, using an independent t test. When data were compared with their own base-line values, paired t tests were used. Differences were considered significant at the $P < 0.05$ level.

Results

Rectal temperature and viremia—Yellow fever caused a slight increase in rectal temperature beginning on PID 3 (Fig 1). The temperature continued to increase and reached a mean peak value of 39.2 C on PID 4, compared with a mean base line of 37.8 C. By PID 5, rectal temperatures decreased, and death became imminent. The presence of viremia corresponded with the febrile period (PID 3 and 4); virus counts were 7.1 to 55 × 10^7 PFU/ml of plasma. Viremia persisted on PID 5 despite a decrease in temperature to 36 C.

Body fluid compartments—Marked alterations of total body, extracellular and intracellular, and interstitial water were not observed after inoculation (Table 1). However, there was a significant expansion of plasma and blood volumes with decreased RBC volume and circulatory PCV. Hemolysis was not observed.

Changes in plasma electrolytes and glucose—Plasma concentrations of Na^+, K^+, and Cl^- of YF-inoculated macaques were not significantly different from that of noninfected controls (Table 2). However, the total amount of circulating K^+ was increased significantly and plasma glucose concentrations decreased during the later stage of YF (Fig 1).

Distribution of tissue water and electrolytes—A significant increase in extracellular K^+ was observed in liver, left ventricular muscle, renal cortex, diaphragm, and skeletal muscle (Table 3). Total K^+ decreased significantly in liver, lungs, and heart. Skin and renal medulla did not reveal

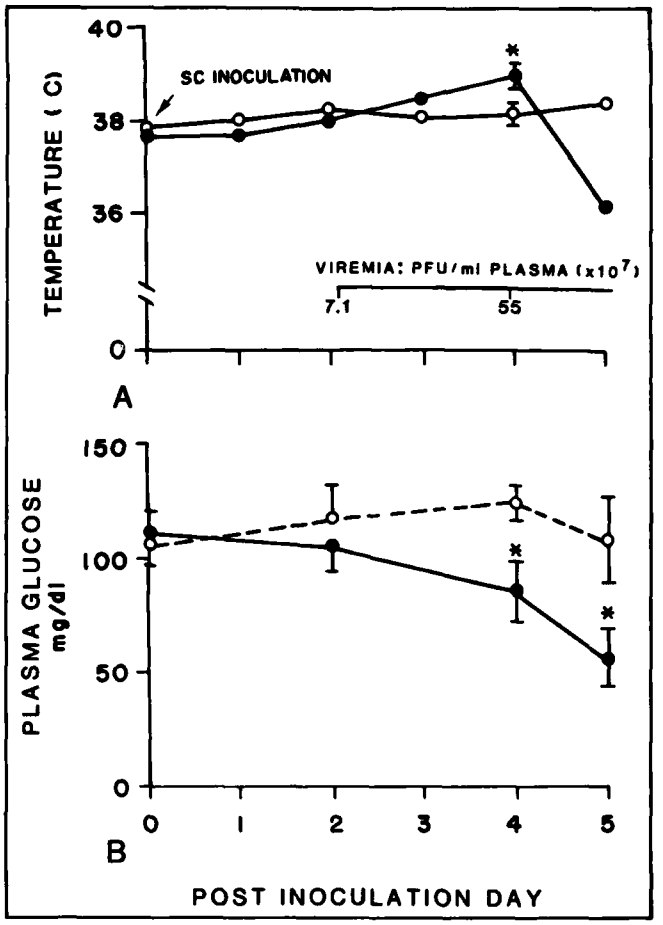

Fig 1—Changes over a 5-day period in rhesus macaques infected by subcutaneous (SC) administration of 40 PFU of YF virus. Rectal temperature and presence of viremia (A) and plasma glucose concentrations (B). ○- - -○ = control (n = 5); ●——● = YF (n = 9); * = $P < 0.05$.

TABLE 1—Changes in body fluid compartments and PCV of rhesus macaques with YF

Variable	Control	YF
Total body water (ml/kg)	663 ± 20	674 ± 27
Extracellular water (ml/kg)	291 ± 9	298 ± 16
Intracellular water (ml/kg)	372 ± 14	376 ± 27
Interstitial water (ml/kg)	245 ± 10	235 ± 17
Plasma volume (ml/kg)	44.3 ± 1.8	60.3 ± 2.9*
RBC volume (ml/kg)	24.0 ± 1.5	19.8 ± 1.0*
Blood volume (ml/kg)	68.2 ± 3.4	80.2 ± 2.6*
PCV (%)	34.8 ± 1.1	26.3 ± 1.8*

* Significantly different ($P < 0.05$) from controls.
Values expressed as means ± SEM.

TABLE 2—Changes in plasma and total circulatory electrolytes in rhesus macaques with YF

Variable	Control	YF
Plasma electrolytes (mEq/L)		
Na^+	152.2 ± 4.1	151.5 ± 5.4
K^+	3.6 ± 0.19	4.84 ± 0.53
Cl^-	113.9 ± 2.7	106.3 ± 2.0
Total circulatory electrolytes (mEq/kg)		
Na^+	6.88 ± 0.43	8.95 ± 0.82
K^+	0.161 ± 0.011	0.282 ± 0.047*
Cl^-	5.16 ± 0.33	6.17 ± 0.48

* Significantly different ($P < 0.05$) from controls.
Values expressed as mean ± SEM.

significant changes in tissue water content or electrolyte concentrations.

The liver had significant increases in total and extracellular water, as well as total and intracellular Na^+. Left ventricular muscle had significantly decreased intracellular and increased extracellular Na^+ values. Renal cortex responded to YF inoculation with intracellular dehydration and extracellular water expansion. These tissue biochemical changes were consistent with the gross pathologic observations of a pale fatty liver, dilated heart, and tensely swollen kidneys typically present in macaques with YF at necropsy.

In the CNS, extracellular K^+ concentration increased significantly with a generally decreased total K^+ (Table 4). Although cerebral cortex showed few changes, extracellular water content and Na^+ concentration increased significantly. Intracellular water decreased in cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord. Intracellular Na^+ was markedly decreased in medulla oblongata.

Changes in tissue minerals and trace metals—The liver had an increase in total Ca^{++} and decreases in Zn^{++}, Fe^{++}, and Cu^{++} (Table 5). The skin revealed increases in Mg^{++} and Zn^{++} values. The Zn^{++} and Fe^{++} concentrations increased in cerebral cortex and medulla oblongata, respectively. Calcium content in left ventricular muscle decreased, compared with that of the control macaques. Tissue minerals and trace metals were apparently unaffected in cerebellum, thalamus-hypothalamus, spinal cord, skeletal muscle, lungs, diaphragm, renal cortex, and renal medulla.

Changes in plasma and tissue lipids—Total cholesterol concentrations of plasma decreased significantly, without marked changes in triglyceride and phospholipid values in YF-infected macaques (Table 6). Total lipids and triglycerides increased in liver. The latter decreased in lungs, cerebral cortex, and spinal cord of infected macaques. The cerebellum had decreased concentrations of phospholipids. Other changes in lipids were not observed.

TABLE 3—Changes in nonnervous tissue water content and electrolyte concentrations of rhesus macaques on PID 5

	Water			Na^+			K^+	
Tissue	Intracellular (g/kg, FFWT)	Extracellular (g/kg, FFWT)	Total (g/kg, FFWT)	Intracellular (mEq/kg, H_2O)	Extracellular (mEq/kg, FFWT)	Total (mEq/kg, FFWT)	Extracellular (mEq/kg, FFWT)	Total (mEq/kg, FFWT)
Liver								
Control	534 ± 9	200 ± 3	734 ± 9	9.8 ± 1.6	31.1 ± 1.0	36.4 ± 0.5	0.73 ± 0.04	82.3 ± 3.4
YF	409 ± 66	400 ± 61*	809 ± 12*	57.1 ± 12.9*	58.9 ± 10	86.1 ± 8.3*	1.87 ± 0.25*	47.0 ± 8.0*
Lungs								
Control	364 ± 35	458 ± 35	822 ± 6	39.7 ± 2.6	70.6 ± 4.1	85.0 ± 2.9	0.53 ± 0.04	59.8 ± 2.9
YF	342 ± 22	479 ± 22	821 ± 2	41.6 ± 13.3	73.8 ± 4.0	87.5 ± 4.5	0.86 ± 0.05	[illegible] ± 2.8*
Heart								
Control	607 ± 10	197 ± 11	804 ± 7	21.8 ± 5.9	31.2 ± 1.6	44.6 ± 3.5	0.75 ± 0.06	93.1 ± 5.4
YF	573 ± 14	229 ± 11	804 ± 3	6.9 ± 1.6*	36.0 ± 1.1*	42.9 ± 1.2	1.13 ± 0.06*	78.7 ± 3.3*
Renal cortex								
Control	563 ± 27	254 ± 24	817 ± 7	38.7 ± 7.3	39.1 ± 3.1	61.1 ± 4.8	0.91 ± 0.06	71.8 ± 3.4
YF	466 ± 29*	355 ± 29*	822 ± 4	25.5 ± 5.7	54.2 ± 3.9*	66.7 ± 2.5	1.67 ± 0.11*	66.6 ± 3.1
Diaphragm								
Control	619 ± 15	147 ± 15	766 ± 4	23.4 ± 6.7	22.8 ± 2.5	37.6 ± 2.7	0.54 ± 0.04	94.8 ± 6.3
YF	584 ± 20	183 ± 17	767 ± 6	19.2 ± 5.1	27.8 ± 2.1	38.2 ± 2.3	0.86 ± 0.05*	74.5 ± 7.2
Muscle								
Control	696 ± 5	80 ± 5	776 ± 7	20.3 ± 5.5	12.4 ± 1.0	26.5 ± 3.1	0.29 ± 0.03	107 ± 8
YF	652 ± 18	113 ± 17	765 ± 5	25.8 ± 6.9	17.2 ± 2.4	34.2 ± 4.5	0.53 ± 0.07*	87.6 ± 4.9

* Significantly different ($P < 0.05$) from controls.
Values expressed as mean ± SEM. FFWT = fat-free wet tissue.

TABLE 4—Changes in CNS tissue water content and electrolyte concentrations of rhesus macaques on PID 5

	Water			Na^+			K^+	
Tissue	Intracellular (g/kg, FFWT)	Extracellular (g/kg, FFWT)	Total (g/kg, FFWT)	Intracellular (mEq/kg, H_2O)	Extracellular (mEq/kg, FFWT)	Total (mEq/kg, FFWT)	Extracellular (mEq/kg, FFWT)	Total (mEq/kg, FFWT)
Cerebral cortex								
Control	596 ± 15	249 ± 18	845 ± 8	50.6 ± 13.4	38.8 ± 3.1	68.4 ± 5.0	0.73 ± 0.05	111 ± 11
YF	539 ± 37	306 ± 35	845 ± 8	28.0 ± 7.1	46.0 ± 4.7	62.8 ± 1.8	1.39 ± 0.11*	97.2 ± 2.2
Cerebellum								
Control	656 ± 23	179 ± 22	835 ± 7	39.7 ± 4.5	27.4 ± 2.8	53.4 ± 3.2	0.64 ± 0.07	110 ± 7
YF	548 ± 25*	291 ± 26*	839 ± 7	26.2 ± 6.5	44.1 ± 3.6*	59.5 ± 2.6	1.35 ± 0.09*	93.4 ± 2.5*
Thalamus-hypothalamus								
Control	595 ± 47	187 ± 43	782 ± 20	53.0 ± 9.3	28.7 ± 6.2	61.0 ± 3.6	0.67 ± 0.15	109 ± 9
YF	504 ± 24	306 ± 26*	810 ± 17	36.4 ± 7.1	46.5 ± 3.2*	65.7 ± 3.6	1.43 ± 0.08*	93.6 ± 2.8
Medulla oblongata								
Control	615 ± 34	213 ± 35	828 ± 17	51.2 ± 12.3	33.4 ± 5.6	66.0 ± 4.0	0.76 ± 0.09	107 ± 8
YF	466 ± 27*	336 ± 24*	802 ± 9	27.7 ± 2.1*	51.0 ± 2.5*	64.0 ± 1.7	1.58 ± 0.06*	96.0 ± 3.3
Spinal cord								
Control	606 ± 26	233 ± 23	839 ± 10	40.2 ± 12.2	36.1 ± 3.5	61.7 ± 6.2	0.85 ± 0.10	102 ± 7
YF	454 ± 38*	356 ± 36*	809 ± 14	37.9 ± 7.3	53.8 ± 4.9*	72.5 ± 3.1	1.63 ± 0.08*	91.0 ± 2.8

* Significantly different ($P < 0.05$) from controls.
Values expressed as mean ± SEM. FFWT = fat-free wet tissue.

TABLE 5—Changes in tissue Ca^{++}, Mg^{++}, and trace metals in rhesus macaques on PID 5

Tissue	Ca^{++} (mEq/kg, FFWT)	Mg^{++} (mEq/kg, FFWT)	Zn^{++} (mg/kg, FFWT)	Fe^{++} (mg/kg, FFWT)	Cu^{++} (mg/kg, FFWT)
Liver					
Control	1.74 ± 0.06	7.36 ± 2.51	46.6 ± 1.4	57.5 ± 7.1	2.08 ± 0.15
YF	3.01 ± 0.34[a]	5.69 ± 0.59	29.0 ± 3.7[b]	38.9 ± 4.0[a]	1.05 ± 0.15[c]
Skin					
Control	3.23 ± 0.32	1.83 ± 0.33	4.93 ± 0.27	25.1 ± 1.8	0.95 ± 0.13
YF	3.32 ± 0.20	4.15 ± 0.51[b]	10.8 ± 0.9[c]	20.6 ± 2.8	1.00 ± 0.10
Cerebral cortex					
Control	2.64 ± 0.62	5.94 ± 1.10	9.91 ± 0.39	28.3 ± 3.3	4.58 ± 0.43
YF	1.90 ± 0.07	6.87 ± 0.38	11.4 ± 0.4[a]	26.9 ± 2.0	4.16 ± 0.18
Medulla oblongata					
Control	1.80 ± 0.05	8.69 ± 0.25	7.06 ± 0.45	12.1 ± 1.2	5.35 ± 0.66
YF	1.95 ± 0.12	8.62 ± 0.37	7.89 ± 0.66	18.5 ± 2.1[a]	4.33 ± 0.28
Heart					
Control	1.81 ± 0.04	7.77 ± 1.11	14.4 ± 2.0	34.8 ± 5.2	3.54 ± 0.6
YF	1.51 ± 0.06[b]	8.41 ± 0.52	17.2 ± 0.6	39.2 ± 2.0	3.87 ± 0.16

Different superscripts (a, b, c) indicate significant difference from controls; [a] ($P < 0.05$), [b] ($P < 0.01$), [c] ($P < 0.001$). Values expressed as mean ± SEM. FFWT = fat-free wet tissue.

TABLE 6—Changes in plasma and tissue lipid concentrations of rhesus macaques after inoculation of YF virus

Tissue	Total cholesterol (mg/100 g of wet tissue)	Triglycerides (mg/100 g of wet tissue)	Phospholipids (g/100 g of wet tissue)	Total lipids (g/kg of wet tissue)
Plasma				
Control	101.4 ± 7.4	35.1 ± 21.9	106 ± 5	ND
YF	68.2 ± 9.6*	44.6 ± 11.1	102 ± 2	ND
Liver				
Control	432.9 ± 29.5	722.7 ± 111	2.8 ± 0.4	38.7 ± 2.4
YF	453.6 ± 47.5	2,165 ± 49.2*	2.6 ± 0.4	87.1 ± 10.8*
Lungs				
Control	486.1 ± 35.1	463.9 ± 77.6	1.56 ± 0.12	11.9 ± 3.1
YF	448.6 ± 46.5	222.6 ± 54.8*	1.41 ± 0.10	12.1 ± 1.8
Cerebral cortex				
Control	1,636 ± 182	500.2 ± 119	3.18 ± 0.59	58.2 ± 5.4
YF	1,709 ± 165	162.8 ± 11*	3.55 ± 0.24	66.8 ± 2.8
Cerebelum				
Control	1,616 ± 208	186.5 ± 39.5	4.03 ± 0.45	78.6 ± 16.0
YF	1,492 ± 124	208.1 ± 45.0	2.94 ± 0.23*	61.5 ± 6.5
Thalamus-hypothalamus				
Control	2,443 ± 173	267 ± 86	5.10 ± 0.48	89.7 ± 14.8
YF	2,954 ± 304	154 ± 12	5.55 ± 0.43	99.7 ± 16.0
Spinal cord				
Control	3,634 ± 226	1,017 ± 206	8.40 ± 0.46	167 ± 4.3
YF	3,927 ± 379	326.0 ± 56*	7.09 ± 0.75	156 ± 11.0

* Significantly different ($P < 0.05$) from controls.
Values expressed as mean ± SEM. ND = not done.

Discussion

Previous studies on YF virus infections in human beings have focused mainly on pathologic changes of the liver.[1,5-8] Although liver damage has been considered to be a major cause of death, the degree of involvement of other vital organs is still not thoroughly understood. Because the rhesus macaque has been shown to be a good animal model for YF,[25] the present study was conducted to demonstrate any changes in water, electrolytes, minerals, trace metals, and lipids in selected tissues of macaques. Demonstrated hypoglycemia and increased extracellular K^+ concentration of the CNS and left ventricular muscle could alter brain and cardiac functions. Further, disturbances of lipid metabolism with YF imply that not only the metabolic functions of the liver, but also of the lung and certain parts of the CNS, were altered.

Degenerative lesions of the heart[26] and kidney[6,27] of rhesus macaques with YF have been demonstrated by other research workers. Lower nephron nephrosis, necrosis of renal cells, and congestion of small vessels (mostly in the medullary zone) were also observed in YF patients.[6,27] The lesions occurring in the heart and kidney during YF represent a structural basis for the alterations of organ functions.[6,8,26,27] Because these macaques could recover from experimental YF with complete regeneration of the liver,[28] pathologic and chemical changes in the liver might be temporary and perhaps noncritical. Biochemical and functional alterations in nonhepatic tissues (heart and kidney) may contribute, in part, to the lethality of YF.

Modifications of brain and spinal cord functions after intracerebral and intraperitoneal inoculations of YF virus have been demonstrated in mice,[29-32] rhesus macaques,[9] and several species of South American monkeys.[33] In infected mice, major findings included cerebral lesions, vascular inflammation, nerve cell damage or degeneration, and morphologic changes in the ventral horn of the spinal cord.[29-32] Acute disseminated encephalomyelitis, necrosis of ganglia, and fatal encephalitis have been observed in monkeys.[9,33]

Early in 1934, Stefanopoulo and Mollaret[34] reported hemiplegia of cerebral origin and optic neuritis in a woman with YF in Africa. These same research workers also recorded the development of encephalitic clinical signs in a rhesus macaque inoculated subcutaneously with attenuated mouse YF virus. In the present study, water and electrolyte metabolism in the CNS was altered as a consequence of YF.

The most striking change was a generalized loss of intracellular water and Na^+ and significant increases in extracellular K^+ in cerebellum, thalamus-hypothalamus complex, medulla oblongata, and spinal cord. These changes were reflected by significant increases in extracellular water and Na^+ in various parts of the CNS. These neurochemical changes are reported to be caused by the invasion of virus directly into the neural cells or indirectly from the nasal mucosa via the olfactory nerve.[32] Another possible mechanism may be that some unknown chemicals or toxins are released from the infected liver and are circulated to the CNS to induce biochemical and functional changes.[35,36]

The relationship between bacterial infection and changes in serum trace metals has been shown to be associated with decreases in Zn^{++} and Fe^{++} and an increase in Cu^{++}.[37,38] Leukocytic endogenous mediator has been considered to be the stimulant for increased hepatic uptake of Zn^{++} and Fe^{++}.[39-41] However, with YF virus infection, liver concentrations of Zn^{++}, Fe^{++}, and Cu^{++} decreased, indicating that trace metal metabolism in the liver is different for bacterial and viral infections. Beisel[38] and Beisel et al[39] reported that in infectious hepatitis, serum Fe values increase (usually 2 to 3 weeks after infection), rather than decrease, compared with that occurring in bacterial infections. The increased content of Zn^{++} was also observed in skin and cerebral cortex of infected macaques. Furthermore, Ca^{++} concentrations increased in liver, but decreased in left ventricular muscle. Because calcium has a key role in regulating cardiac contractile force,[42] its loss from heart may be associated with decreased contractility.

In the present study, YF-infected rhesus macaques showed a marked expansion of plasma and blood volumes with significant decreases in RBC volume and circulatory PCV. Because the increase in plasma volume exceeded the magnitude of decreased RBC volume, the blood volume was significantly increased despite a decrease in volume of RBC mass. Furthermore, changes in total body, extracellular and intracellular, and interstitial water were not significant. Plasma concentrations of Na^+, K^+, and Cl^- were also changed insignificantly. The mechanism for fluid changes with viral hepatitis was believed to be a failure of hepatic inactivation of antidiuretic hormone.[43] The association of viral hepatitis and renal disease has also been reported[44-46] as a result of immunologic reactions occurring in the kidney. With acute viral hepatitis, the increased secretion of aldosterone and impaired renal functions (decreases in renal blood flow and glomerular filtration rate) would cause Na^+ and water retention and disturbances of electrolyte metabolism.[47]

Although the liver was a major target organ for YF virus, the CNS, heart, lungs, kidneys, skeletal muscle, and diaphragm were also affected biochemically and physiologically. These findings indicate that YF is a multiorgan disease. If the extracellular fluid compartment is measured alone, without searching for the fluid volume and electrolyte changes at the organ level, the most important responses of fluid movement in certain tissues may be overlooked during YF. Despite the fact that total extracellular fluid volume was essentially unaltered in infected macaques, independent changes in tissue water and electrolytes were demonstrated. This discrepancy may be caused by the use of an insensitive method for measuring total extracellular fluid compartment (ie, thiocyanate space), when extracellular fluid volumes were changed in few selected tissues.

By using the same techniques for measuring tissue distribution of water and electrolytes, normal base-line values from a previous study[18] and the present work are similar. The consistent data indicate that the applied methods of analyzing tissue fluid volume and electrolytes are valid and reproducible. When these techniques were used for the study of pathogenesis of Rocky Mountain spotted fever in rhesus macaques, intracellular overhydration in the medulla oblongata was observed.[48] In contrast, YF virus produced intracellular dehydration of the medulla oblongata, cerebellum, and spinal cord. Cellular fluid loss in brain and spinal cord may contribute partially to death, as a result of central cardiopulmonary depression in YF-infected macaques.

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